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TECHNICAL REVIEW AND APPROVAL

AFAMRL-TR-81-81

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals, "Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The Respiratory Toxicology research programs conducted at the Toxic Hazards Research Unit (THRU), Wright-Patterson Air Force Base by the University of California, Irvine are reviewed in this annual report. Preliminary results are presented of studies concerning: 1) the effect of propylene glycol dinitrate on the cardiovascular system in the dog; 2) the evaluation of pulmonary clearance after exposure to 1.2 ppm of ozone, and; 3) the evaluation of small airway test sensitivity in enzyme induced emphysema.

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PREFACE

This is the first annual report of the Respiratory Toxicology broadening program of the Toxic Hazards Research Unit (THRU) and concerns work performed by the Department of Community and Environmental Medicine of the University of California, Irvine on behalf of the Air Force under Contract Number F33615-80-C-0512. K. C. Back, Ph.D., Chief of the Toxicology Branch and M. K. Pinkerton were the contract technical monitors for the Air Force Aerospace Medical Research Laboratory. Paul E. Newton, Ph.D. served as head of the Respiratory Toxicology program and acknowledgement is gratefully made for the research assistance of Chris Pfledderer and Stanley Erk, Sr., the advice and support of T. T. Crocker, M.D., J. D. MacEwen, Ph.D. and E. H. Vernot, the assistance of C. E. Johnson and the entire engineering staff at THRU in the construction and set up of this program's research facilities and the contributions of J. L. Scheerschmidt and J. A. Sizemore in the preparation of this report.

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SECTION I
INTRODUCTION

The lung is a major portal of entry into the body for most chemical pollutants and in many cases becomes the target organ for toxicity. While histopathologic lesions seen after death may describe the severity of toxicologic change, a necessary complement to these descriptive-toxicology findings is an understanding of the onset and mechanism of pulmonary injury from inhaled chemicals. Consequently, a pulmonary toxicology research laboratory adjacent to the THRU exposure facilities has been established to develop and install methods for the evaluation of direct injury to the lung as measured by tests of the gas exchange capability of the lung and the ability of the lung to protect itself from inhaled particles. This laboratory will also conduct toxicokinetic studies that measure pulmonary uptake and excretion of inhaled gases and vapor, both at rest and during exercise.

PULMONARY GAS EXCHANGE

It is the intent of this program to develop tests in the areas of: (a) pulmonary mechanics (pressure-volume loops, lung volumes); (b) pulmonary dynamics (density dependent maximum expired flows, resistance and compliance); (c) distribution of ventilation (N_2 washout); (d) membrane diffusion; (e) respiratory control center, and; (f) the ultimate test of lung efficacy, arterial oxygenation ((A-a)P_{O_2}, pH, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, R and Hb).

Because of the concern of an adverse effect not only on healthy people but also on more susceptible ones, possible effects not seen in healthy animals and/or synergistic effects will be looked for in animals already compromised with human (analogous) disease states, such as enzyme induced emphysema. Since exercise increases the total dose inhaled and may have other synergistic effects, animals will also be tested at rest and under controlled exercise conditions.

PULMONARY DEFENSE

The clearance efficiency of particles deposited in the respiratory system is of major interest when considering pulmonary defense mechanisms. Any decrease in clearance efficiency will consequently increase the deposited particles' retention time within the respiratory system and if these particles are toxic or infectious in nature, will increase the susceptibility to injury.

Inhalation of an aerosol of 1-3 μm diameter ^{51}Cr-labelled particles provides a deposition of particles throughout the lung and allows monitoring of pulmonary clearance, using external radiation detectors. Measuring the activity excreted in fecal samples, taken at fixed intervals, monitors the short-term clearance capability via the mucociliary ladder. Measuring thoracic activity monitors the long-term clearance of the deep lung.

TOXICOKINETICS

The measurement of inhaled and exhaled concentrations on a breath by breath or steady state basis gives data on uptake of inhaled compounds and the resulting body burden to be correlated with toxic effects. The influence of exercise upon the uptake of a compound will be investigated using: an air conditioned animal treadmill to prevent panting during exercise; a mass spectrometer with both gas and blood or tissue catheters; a gas chromatograph and blood gas analyzer. While the setup will be for the dog, with the incorporation of very small breathing valves and intense animal training, experiments using small animals will also be attempted.

SECTION II
RESEARCH PROGRAM

For the 1980-81 contract year, research was initiated in each of the above 3 research areas. Due to delays incurred in the receipt, setup, and check out of equipment, none of these studies has been completed to date. Progress reports for these studies are included as part of this report.

THE TOXICOKINETIC STUDY OF 1,2-PROPANEDIOL DINITRATE (PGDN)

Research Aims

It is the purpose of this study to (1) define the kinetics of the uptake and elimination of PGDN in anesthetized dogs via iv injection, inhalation through an endotracheal tube, inhalation through a mask, and inhalation through a mask in the awake dog at rest and during exercise; and (2) correlate the physiologic effects produced by PGDN with arterial PGDN and PGDN metabolite concentrations. These data and information from additional on-going studies will be used to construct a toxicokinetic profile. This model will aid in the projection of dose-response data to other dose levels and in extrapolation of relevant toxicologic observations to the human.

Significant Literature

1,2-Propanediol dinitrate has several synonyms which have appeared in the literature including propylene glycol dinitrate, PGDN, and propylene dinitrate. Its chemical formula is $CH_3CHONO_2CH_2ONO_2$ with a molecular weight of 166.11.

PGDN was originally considered as a replacement for ethylene glycol dinitrate (EGDN) in the manufacture of antifreeze dynamite after it was found that EGDN could lead to vasodilation problems and also to sudden death of workers (Kylin et al., 1966).

Its primary use now is as a fuel component in torpedoes and other weapon systems for the U.S. Navy. PGDN is the major constituent of Otto Fuel II (OFII) to which a desensitizing agent and a stabilizer have also been added (Navy Environmental Health Bulletin

and JANNAF, 1969). Studies have shown that OFII is neither water soluble nor biodegradable. PGDN toxicity becomes of some importance since wastewater from torpedo refueling facilities contains small amounts of OFII (Kessick et al., 1978).

Although the vapor pressure of OFII is quite low, conditions exist in which vapor concentrations of 100 to 150 ppm can occur thereby requiring information on the inhalation toxicity of PGDN.

The studies concluded to date have touched three areas of the toxicology of PGDN. These areas are descriptive, metabolic mechanisms, and behavioral.

Kylin et al. (1966) completed the first work by determining the ip LD_{50} in female albino mice for PGDN as well as EGDN and nitroglycerine (GTN). PGDN and EGDN had similar values, 930 mg/kg and 800 mg/kg, respectively, whereas the GTN was approximately four times more toxic at 194 mg/kg. Limited iv elimination studies were also carried out on rabbits indicating that the initial breakdown was extremely rapid (within minutes). There was no apparent rate difference among the three tested materials; however, an "unexplained difference" occurred between arterial and venous blood concentrations in each case.

A more thorough study was conducted by Clark and Litchfield (1969). The LD_{50} was calculated for PGDN on several species and routes as listed in Table 1. The pharmacologic effect of arterial blood pressure drop and formation of methemoglobin was also correlated to dosage. Here it was found in the rat that the maximum blood pressure depression was concurrent with the maximum level of PGDN in the blood. Additionally, the appearance and disappearance of metabolites in the blood stream were followed both in vitro and in vivo. Results indicated that, as with other nitrate esters of polyhydric alcohols, PGDN was broken down to inorganic nitrate and nitrite as well as the mononitrate esters of the parent compound. Litchfield (1971) further indicated that the propylene glycol 2-mononitrate predominated in the blood and that there was some reaction favoring the formation of this isomer over the propylene glycol 1-mononitrate.

TABLE 1. ACUTE LD_{50} OF PGDN*

Species	Sex	Route of Administration	LD_{50} (mg/kg)
Rat	F	PO	1190
Rat	F	SC	463
Rat	M	SC	524
Mouse	F	SC	1208
Cat	F	SC	200-300

*(Clark and Litchfield, 1969)

Inhalation studies involving animals were conducted by Jones et al. (1972). Male Sprague Dawley rats were exposed to 65 mg/m^3 (9.57 ppm) PGDN vapor 7 hr/day, 5 days/wk, for a total of 30 exposures. No toxic effects were seen. Hematologic parameters were unchanged and histopathologic examination of tissue did not show any exposure related lesions. Continuous 90-day inhalation exposures of rats, guinea pigs, monkeys, and dogs were conducted at three PGDN concentrations: 67 mg/m^3 (9.87 ppm); 108 mg/m^3 (15.9 ppm); and 236 mg/m^3 (34.76 ppm). Rate of body weight gain was unaffected by PGDN exposure. Decreases in hemoglobin (63%) and hematocrit (37%) were observed in dogs exposed to 236 mg/m^3 PGDN. Methemoglobin levels were elevated in all species with dogs and monkeys showing the most dramatic increases. Iron-positive deposits were present in the livers, spleens, and kidneys of dogs and monkeys exposed to 236 mg/m^3 PGDN. Fatty changes in the livers and kidneys were also noted in the animals exposed to PGDN vapor.

The chemical effects of PGDN were investigated by Andersen and Smith (1973). Their observations were based on in vitro studies of both human and rat hemolysates and intact red blood cells. The reaction of PGDN was found to be molecular rather than enzymatic and approximately first order. The stoichiometry of the hemolysate was 1.5 heme groups oxidized per ester bond broken and 1.9 to 2.3 moles heme oxidized per mole of reacted ester while using whole cells.

Monoamine oxidase (MAO) activity in rabbit livers (in vitro) was examined in light of the assertion that certain MAO inhibitors exert a therapeutic (vasodilation) effect on angina pectoris (Kalin and Kylin, 1969). It was found that PGDN, EGDN and GTN all were competitive inhibitors of MAO activity, while their metabolites showed no such effect. The conclusion was then drawn that a tolerance mechanism occurs upon chronic EGDN exposure which, when exposure stops, causes an angina-like effect. In severe cases, vascular spasm may then be followed by death when exposure ceases.

Behavorial testing in conjunction with exposure to PGDN has been reported by several authors. Jones et al. (1972) trained four monkeys to perform a visual discrimination test (VDT). They were then injected with a 4 mg/kg dose, allowed one week of rest and then reinjected with a 7 mg/kg dose. Observations while using the VDT at one hour preinjection, immediately after injection, and for three one hour periods postinjection showed minimal behavorial effects. Another set of monkeys were trained on the VDT or on a visual acuity threshold test (VATT) before being continuously exposed to 262 mg/m^3 (38.5 ppm) for 90 days. Testing each week indicated no changes in avoidance behavior patterns as revealed by the VDT or VATT.

A ceiling threshold limit value (TLV) of 0.2 ppm PGDN vapor has been established (Navy Environmental Health Bulletin). In 1974, Stewart et al., reported a series of human inhalation exposures to PGDN vapor at a concentration close to the TLV. Headaches occurred in a majority of the individuals exposed to 0.2 ppm PGDN for 4 hours or more. Repetitive exposure to this concentration produced a tolerance to the headaches. The development of tolerance has

previously been described for other organic nitrate compounds. No alteration in blood chemistry values occurred in humans after a single 8 hour exposure or repetitive 8 hour exposures to 0.2 ppm PGDN vapor. No PGDN was detected at any time during blood analysis.

Although some of the toxic effects of PGDN on rats, monkeys, and rabbits have been described, little work has been accomplished using dogs as the animal model even though they are quite susceptible to methemoglobinemia. No effort has been made to elucidate the pharmacokinetic factors in this species. It is the purpose of this study to define the kinetics of elimination of PGDN after acute inhalation exposures. These values will also be correlated with the pharmacologic action of blood pressure depression and methemoglobin formation. Additional kinetic data will be obtained by evaluating PGDN uptake and elimination at increased metabolic rates.

Methods

Test Agent

The test agent is 1,2-propanediol dinitrate (PGDN). It is supplied as a mixture in methanol (40g PGDN:500 ml). The physical properties of the pure material are listed in Table 2.

TABLE 2. PHYSICAL PROPERTIES OF PGDN*

Density	1.4 g/ml
Vapor Pressure	0.07 mm Hg (22.5°C)
Boiling Point	92°C (10 mm Hg)
Color	Colorless
Solubility	0.130 g/100 ml H_2O

*(Clark and Litchfield, 1969)

Purity of both the mixture and washed PGDN will be assayed via a Waters high pressure liquid chromatograph utilizing refractive index and ultraviolet (254 nm) detectors. Conditions of the instrument will be as follows:

Column:	µBondapak C-18
Injection Volume:	1.0 µl
Solvents:	Methanol/Water
Ratio:	90/10
Flow:	0.6 ml/min
Pressure:	700 PSIG

Experimental Approach

Four dogs will be exposed to three doses of PGDN using 4 different types of administration. Exposure levels will be based on blood pressure, methemoglobin and PGDN data obtained from the preliminary studies. The route of administration will be: iv; inhalation via an intubation tube; inhalation via a mask while either

anesthetized or awake (awake being while resting and during exercise). Each dog will be exposed only once to PGDN.

Animals will be housed at the Veterinary Sciences Division Building 838 (Vivarium) until needed. Experimental procedures will be carried out in Room #122, Building 79.

Animal Data

Male beagle dogs of approximately one year of age will be used in the study. The animals will be quarantined for a minimum of one month during which time background clinical data will be collected and analyzed.

Food, Purina Lab Canine Diet #5006, will be available ad libitum until 24 hours predosing.

Water will be made available ad libitum. Water hardness, measured as calcium carbonate, is not to exceed 17 ppm.

Animals will be caged in conformance with the Institute of Laboratory Animal Resource standards. All cage areas will be cleaned daily.

Contaminant Generation and Monitoring

Introduction System

The contaminant introduction system for the inhalation studies will consist of an agitated supply of PGDN maintained at a constant temperature with a controlled air sweep to carry the necessary PGDN vapor to the air mask input line. The generation apparatus will be contained in a ventilated hood. Concentration control will be by varying PGDN temperature and air flow rate.

Monitoring

Analysis of the concentration of PGDN in inhaled and exhaled air will be accomplished utilizing a Balzers 511 Mass Spectrometer. The PGDN fragmentation ion, m/e 90, will be monitored and recorded. This ion shows no interference from exhaled breath and has been shown to have more than adequate response to PGDN in the 35 ppm concentration range. Continuous sampling of the gases will be through a 1/16" O.D. stainless steel capillary tube. A switching system will be built which will first sample from the contaminant introduction system for a selected time period and then sample the exhaled gases which will be collected in a mylar bag.

Calibration of the mass spectrometer will be accomplished by sampling gas standards made from measured amounts of pure PGDN. The gas standards will also be contained in mylar bags.

Analysis of PGDN and possible mononitrate metabolites in the blood will be accomplished via gas chromatography. Blood samples

will be drawn from an artery and then extracted with diethyl ether. The ether extract will be concentrated and dried. Aliquots will be separated using a 3M column of UC-W98 on Chromosorb W-HP. The detector of choice will be electron capture with a $_{63}Ni$ source and a mixture of 5% methane: 95% argon for a carrier gas.

Calibration of the GC will use standards that have been prepared and extracted from blood. An internal standard will be used if an appropriate one can be found. Initial efforts along this line have shown GTN to be less than satisfactory while we were unable to obtain EGDN, another possible internal standard.

Additional Analyses

The method of Rodkey et al. (1979) for analyses of carboxyhemoglobin and methemoglobin has been set up and standardized using dog blood for standards. Analysis of blood gases (P_{CO_2} and P_{O_2}) and pH will also be tested at this time to show that blood acid-base balance and oxygenation remain within acceptable limits, i.e., pH 7.32-7.45, P_{CO_2} 30-50 torr and P_{O_2} >75 torr. A self-calibrating (every 1.75 hour) Radiometer Copenhagen Model ABL-2 Acid-Base Laboratory will be utilized for these determinations. Quality control blood samples (made with standard gases on a Dynex tonometer) will be analyzed monthly.

Treadmill

An animal treadmill (Quinton) equipped with a completely enclosed air conditioned box (P. Reischl, personal communication) will be used to exercise the dogs and produce increased metabolic and uptake rates. The air conditioned box's air temperature is controlled by a computer (HP9845T) to a temperature that keeps the continuously measured rectal temperature at a pre-exercise level and thus prevents panting. The dogs are trained to breathe through a tight fitting molded mask containing a modified Hans Rudolph valve (P. Reischl, personal communication). Inspired and expired gases are conducted through heated tubes connected to the dog mask. Training sessions will be conducted prior to exposure days to acquaint the dog with the box and treadmill procedures.

Technique

Several mock runs will be made using spare dogs rejected from other studies. This will be done to coordinate the anesthetic procedures, mass spectrometer analysis, methemoglobin and blood gas analyses, blood pressure and body temperature control. A three-fold anesthetic approach will be used: (1) lidocaine hydrochloride for use as a local during the cut down procedure; (2) α-chloralose will be used to induce sleep without depression of respiration (VanStee, 1980); and (3) morphine sulfate will be used to produce analgesia. The α-chloralose will be infused in a 5% dextrose in Ringers solution at a dose of 110 mg/kg (Lumb and Jones, 1973). Two dogs, designated as controls, will be subjected to the entire procedure without PGDN. Data from these runs will be used as baseline

information. An additional two control dogs will serve to produce treadmill baseline data while varying ventilation (derived from V_{O_2}) to 2X and 3X that of the resting state. Inhalation exposure concentrations and times will be based on iv elimination studies in progress.

Observations and Tests

Pre-Dosing

Prior to exposure, a minimum of three body weights and three jugular vein blood samples will be obtained at intervals not less than one week apart. Monthly weights and blood samples will be taken until the animal is utilized.

Observation

Each animal will be observed continuously from the start of the anesthetization period until termination of that experiment. Body core temperature and blood pressure will be recorded. Core temperature will be maintained through external heat sources.

Blood

22.0 ml clinical blood samples will be drawn from the jugular vein. Clinical determinations will be those as listed in Table 3. All tests will be performed as soon as possible after collection to minimize sample storage time. Additional 3.0 ml heparinized samples will be taken during the experiment for PGDN P_{CO_2}, P_{O_2}, pH, methemoglobin, and carboxyhemoglobin determinations.

TABLE 3. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON DOGS INFUSED WITH PGDN

Hematology	Chemistry
Hematocrit[a]	Sodium[b]
Hemoglobin[a]	Potassium[b]
Total RBC[a]	Calcium[c]
Total WBC[a]	Albumin/Globulin[c]
Differentials	Total Protein[c]
Mean Corpuscular Volume (MCV)[a]	Glucose[c]
Mean Corpuscular Hemoglobin (MCH)	Alkaline Phosphatase[c]
Mean Corpuscular Hemoglobin Concentration (MCHC)	SGPT[c]
	SGOT[c]
	Bilirubin[c]
	Creatinine[c]
	BUN[c]

a Determined on a Hycel HC-500 Counter
b Determined on an Instrument Laboratory 443 Flamephotometer
c Determined on a DuPont Automatic Clinical Analyzer

Necropsy

The test animal which is used in each experiment will be necropsied as soon as possible in order to determine that there are no gross abnormalities. The necropsy is defined as a gross examination of all body organs including body orifices. The tissues listed in Table 4 will be sampled, fixed in formalin and retained for histopathologic examination if desired at a later time. Organ weights for liver, kidney, spleen, heart and lungs will also be taken at the time of necropsy.

TABLE 4. TISSUES SAMPLED FROM ANIMALS EXPOSED TO PGDN VAPOR

Gross Lesions	Stomach
Tissue Masses or Suspect Tumors and Regional Lymph Nodes	Pituitary
Lungs and Bronchi	Duodenum
Heart	Ileum
Thyroids	Colon
Parathyroids	Liver
Mesenteric Lymph Nodes	Spleen
Thymus	Kidneys
Gall Bladder	Bladder
Pancreas	Brain
Adrenals	

Data Analysis

Data from routine animal weighing, hematology, blood chemistry, and organ weighing will be analyzed for statistical significance using the t-test. Dose response curves will be generated for Plasma PGDN vs. Time; Plasma PGDN vs. Blood Pressure; and Plasma PGDN vs. Methemoglobin Values. The order of elimination of PGDN and its half-life will be determined. Additionally, Km and Vmax values will be calculated from Lineweaver-Burk plots. These values will be compared as to inhalation and iv studies and the effects of increased uptake due to increased workload. Pulmonary uptake loads will be determined by two methods, (1) difference between vapor concentrations in inhaled and mixed-exhaled air and (2) difference between inhaled vapor quantity and arterial blood levels. It is expected that at this point data will be generated concerning possible protein binding effects.

Test Agent Handling Procedure

The methanol:PGDN mixture and washed PGDN will be stored in a Class III fume hood in Room #122, Building 79. The containers will be labelled as follows:

Material Name (PGDN or Mixture)
Date Opened
Person Receiving or Preparing the Compound
Hazards: Flammable
Toxic
May be Shock Sensitive

The methanol will be removed from the mixture by blowing helium over the surface of the liquid. Only amounts needed will be purified. This procedure will be performed in a fume hood. Protective equipment consisting of gloves, lab coat, and goggles or face shield will be worn during this procedure.

Progress Report

This study has been initiated and range-finding studies with injections of 4 and 40 mg PGDN per kg body weight have been conducted. The 40 mg/kg dose was selected as being the approximate dose for a 10 kg dog from an exposure to 35 ppm for 6 hours with a ventilation rate of 5 liter/min and 100% retention. The 35 ppm level was selected because it is currently being studied in a dome study at THRU. A 30 minute calculated dose would be near 4 mg/kg and this dose was selected as the second level in these range-finding studies. Figures 1, 2 and 3 show the effect of an iv injection of 4 or 40 mg PGDN per kg body weight dissolved with 1 drop of Tween 80 in 20 ml heparinized saline on arterial blood pressure and heart rate in the dog. Subsequent studies have shown, however, that these effects were not due to PGDN but to the Tween 80 used to dissolve the PGDN into the heparinized saline. Tween 80 had been selected after attempts to dissolve PGDN in plasma, physiologic saline, ethanol, or acetone were unsuccessful even after it was heated and ultrasonicated. The addition of 1 drop of Tween 80 allowed the PGDN to be mixed with saline.

However, a control study where 1 drop of Tween 80 in 10 ml heparinized saline was injected produced the results shown in Figures 4 and 5. Therefore, the effects seen in Figures 1-3 which were previously attributed to PGDN are apparently due to the Tween 80. This effect was seen after two different injections of Tween 80. The second injection was done to confirm the Tween 80 effect using new tubes and freshly prepared solutions to preclude some unknown contamination from PGDN. Analysis by gas chromatography showed no measurable PGDN in the Tween 80 solution.

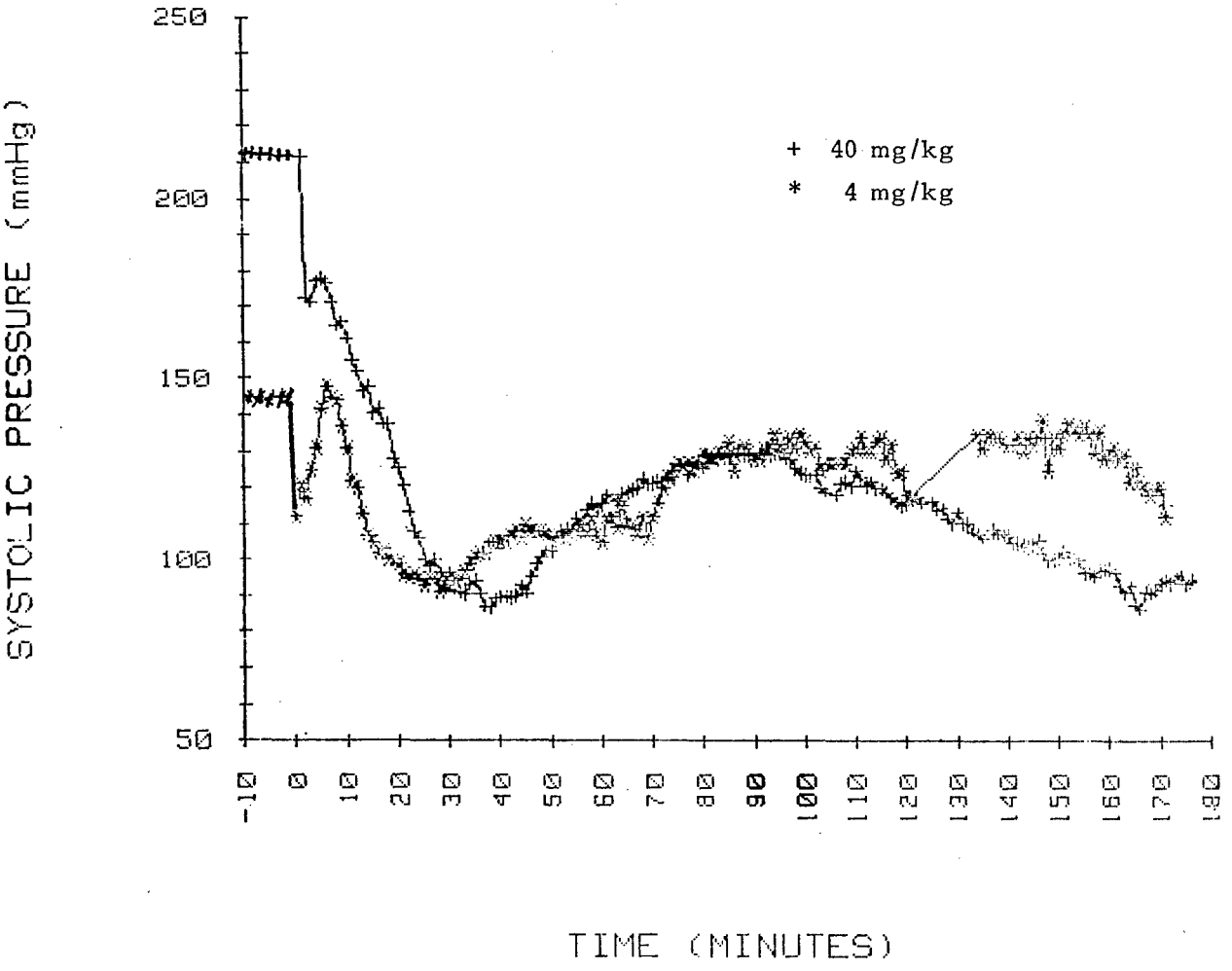


Figure 1. The effect of 4 or 40 mg/kg PGDN dissolved with 1 or 3 drops Tween 80, respectively, in 10 ml heparinized saline on systolic pressure in the dog. Subsequent experiments have shown that the above effects were not due to PGDN but instead were primarily due to the Tween 80. The apparent dose response was due to 3 drops of Tween 80 being used for the 40 mg/kg experiment and 1 drop of Tween 80 being used for the 4 mg/kg experiment.

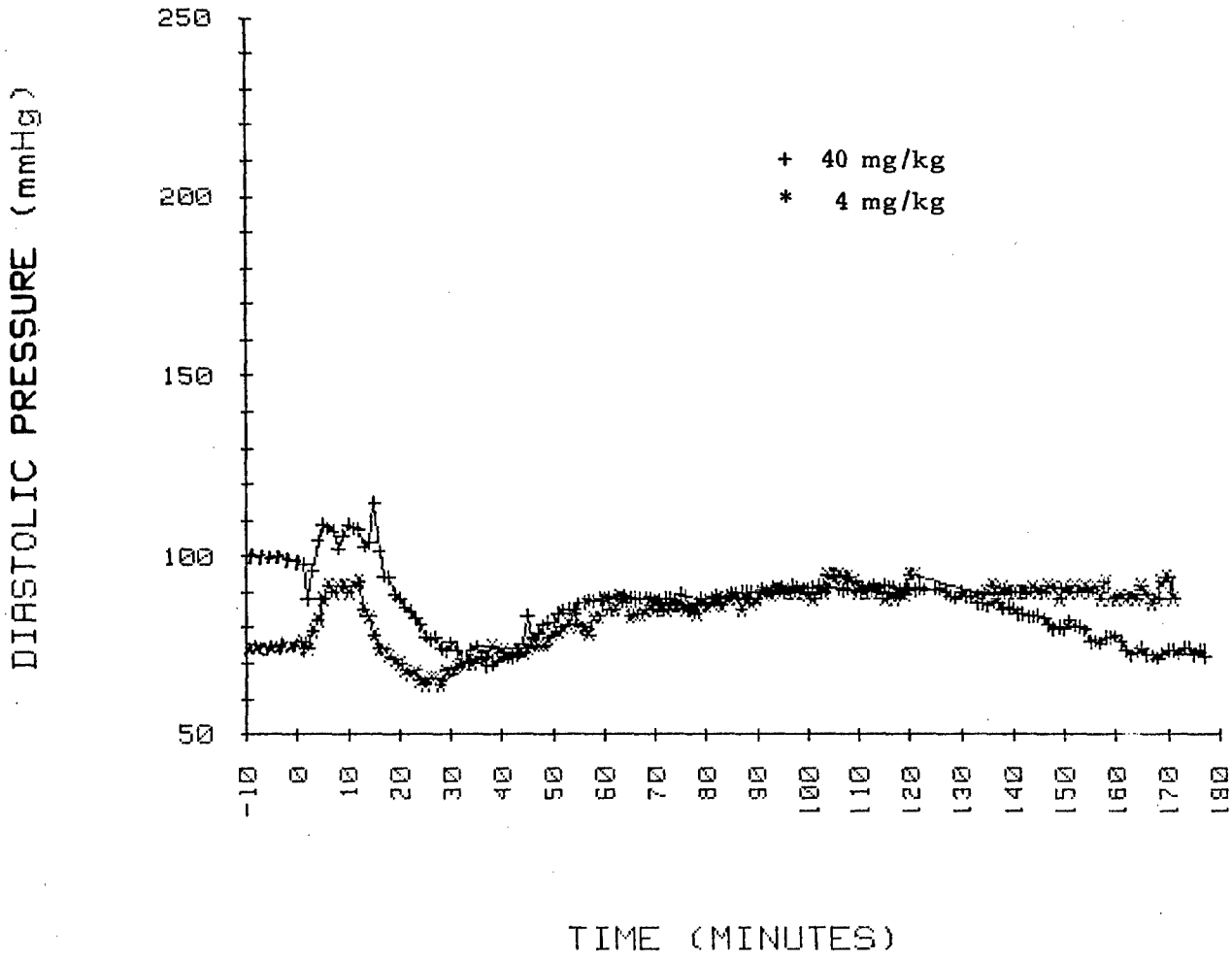


Figure 2. The effect of 4 or 40 mg/kg PGDN dissolved with 1 or 3 drops Tween 80, respectively, in 10 ml heparinized saline on diastolic pressure in the dog. Subsequent experiments have shown that the above effects were not due to PGDN but instead were primarily due to the Tween 80. The apparent dose response was due to 3 drops of Tween 80 being used for the 40 mg/kg experiment and 1 drop of Tween 80 being used for the 4 mg/kg experiment.

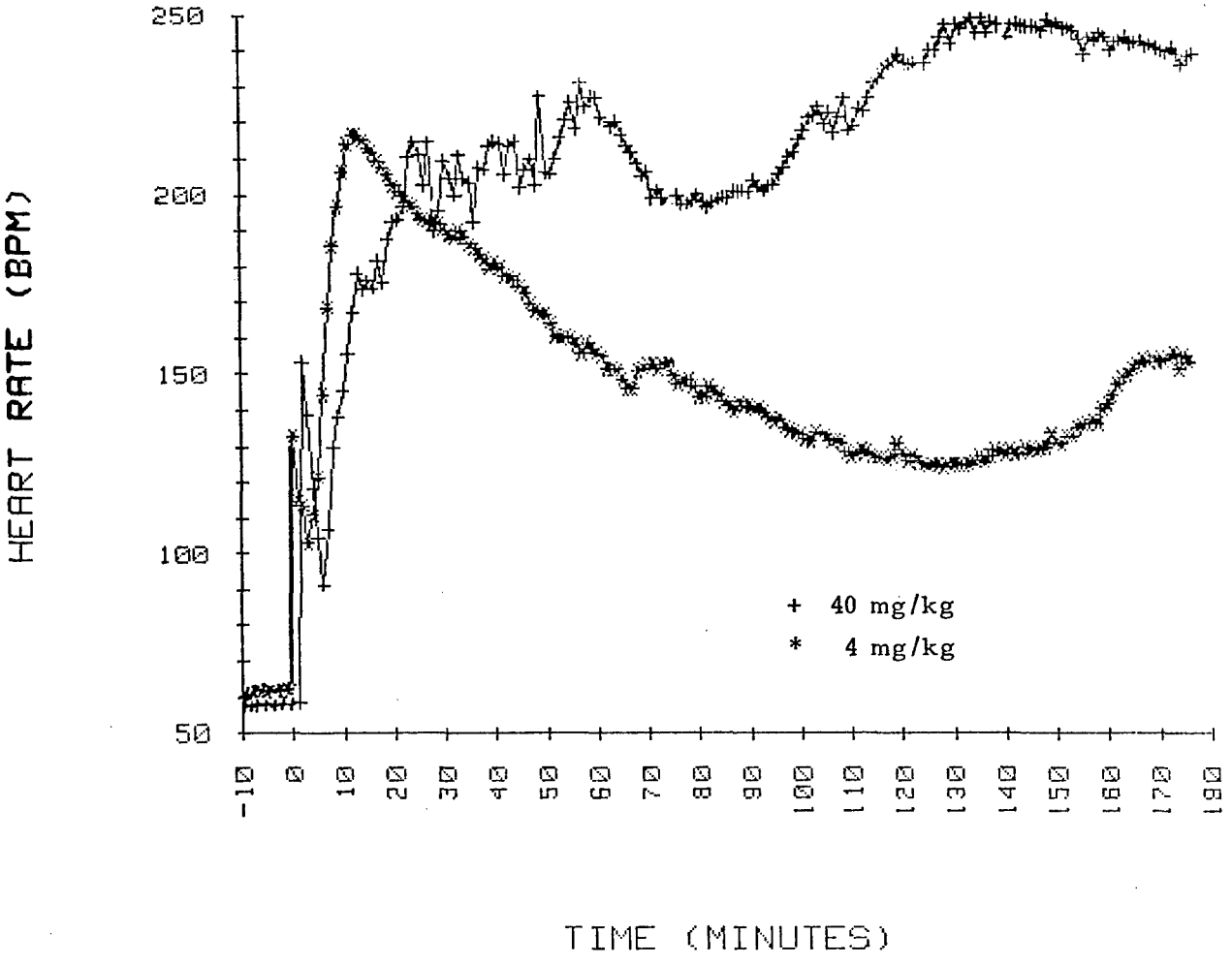
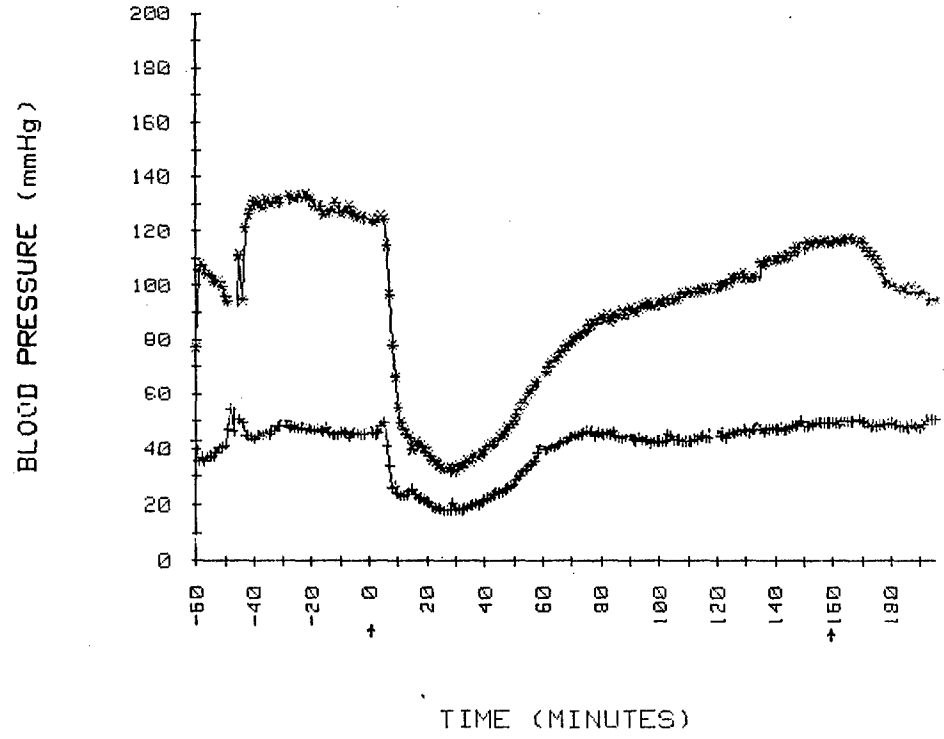
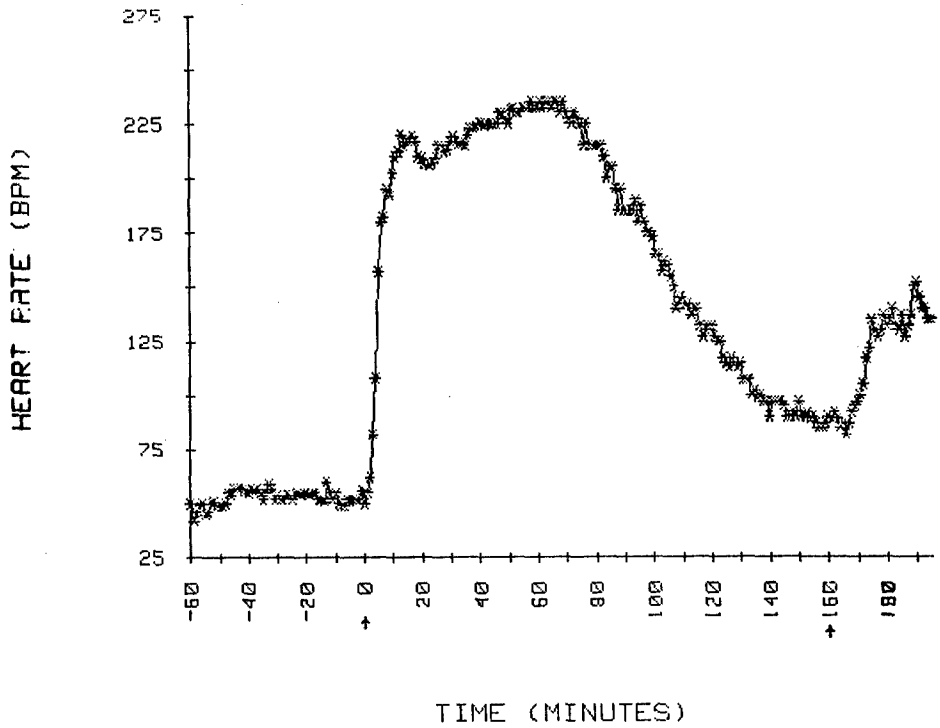


Figure 3. The effect of 4 or 40 mg/kg PGDN dissolved with 1 or 3 drops Tween 80, respectively, in 10 ml heparinized saline on heart rate in the dog. Subsequent experiments have shown that the above effects were not due to PGDN but instead were primarily due to the Tween 80. The apparent dose response was due to 3 drops of Tween 80 being used for the 40 mg/kg experiment and 1 drop of Tween 80 being used for the 4 mg/kg experiment.



† = Injection of 1 drop of Tween 80 in 10 ml of heparinized saline

Figure 4. The effect of Tween 80 on blood pressure in the dog.



† = Injection of 1 drop of Tween 80 in 10 ml of heparinized saline.

Figure 5. The effect of Tween 80 on heart rate in the dog.

These injections of Tween 80 also produced an elevated T wave in the dog's electrocardiogram (Figure 6). Since elevated potassium could also produce a similar effect and it was theorized that the surfactant activity of Tween 80 might have altered the cellular membrane such that electrolyte changes could occur, blood analyses were performed on the control (Tween 80 only) dog. Table 5 shows the results of these analyses. Whereas baseline blood values on this dog drawn 1 week prior to the exposure were all within normal limits, the postinjection blood sample showed abnormally low potassium, albumin, and total bilirubin and an abnormally high glucose level. Therefore, the elevated T wave cannot be explained by an elevated potassium level. However, a potassium alteration did occur.

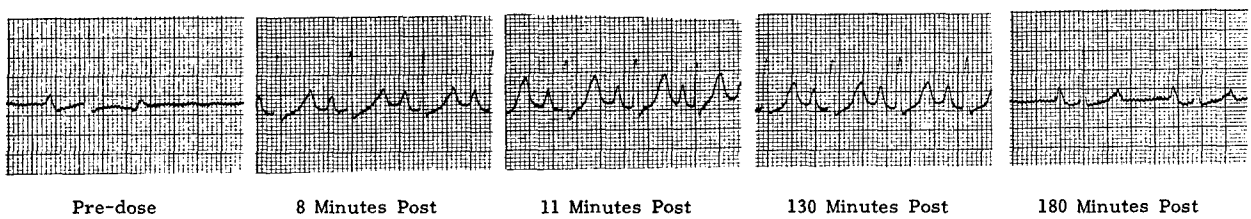


Figure 6. Effect of Tween 80 on the Lead II electrocardiogram in the dog.

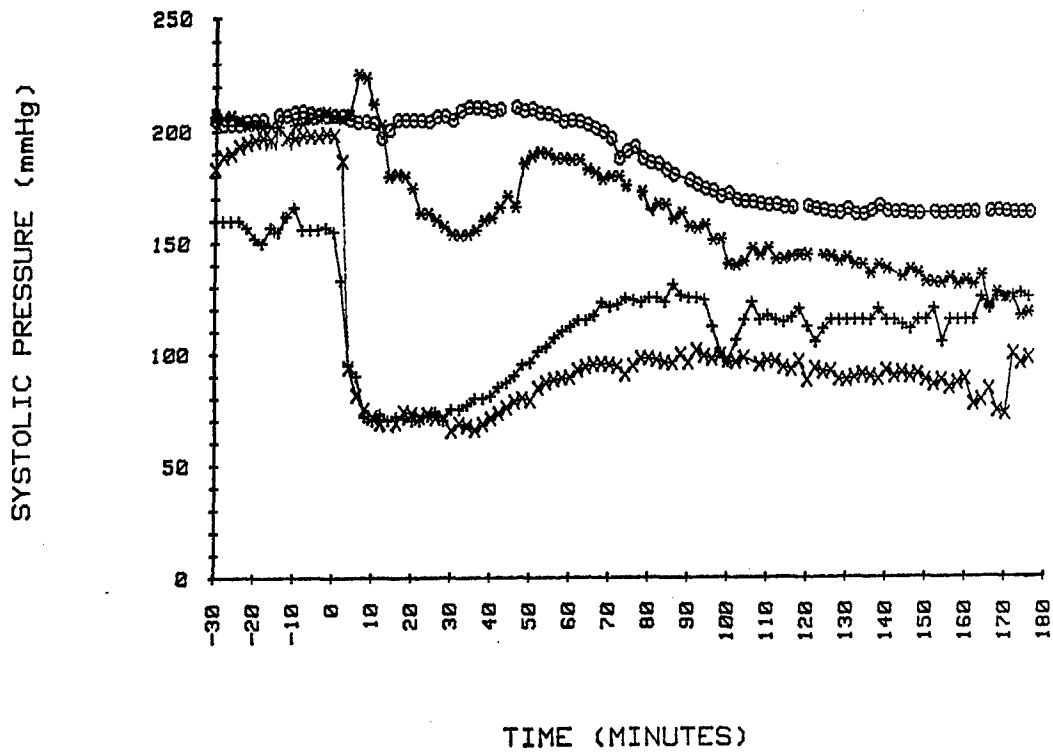


Figure 7. The effect of an iv injection of 10 ml of heparinized saline containing 0 (0), 2 (+), 4 (*) or 6 (X) mg/kg of Tween 80 on systolic pressure in the dog.

TABLE 5. BLOOD ANALYSES FOR BASELINE AND 2 HOURS POST INJECTION OF 1 DROP OF TWEEN 80 IN 20 ML HEPARINIZED SALINE

	Baseline	Post Injection	Lower-Upper Confidence Level
RBC, $x10^6/mm^3$	6.38	5.65	5.4 - 8.8
WBC, $x10^3/mm^3$	14.5	9.5	3.6 - 22.1
HCT, %	42	41	37 - 56
HGB, g/dl	15.3	14.3	12.6 - 19
Bands, %	12	6	---
Neutrophils, %	61	74	---
Lymphocytes, %	27	20	---
Sodium, meq/L	148	156	141 - 161
Potassium, meq/L	4.8	3.4*	4.1 - 5.6
Calcium, mg/dl	9.8	9.6	9.5 - 12
Glucose, mg/dl	97	284*	81 - 133
Total Protein, g/dl	6.1	5.2	4.9 - 7.3
Albumin, g/dl	3.2	2.3*	2.8 - 4.2
Globulin, g/dl	2.9	2.9	1.4 - 3.8
SGOT, IU/L	39	47	15 - 50
SGPT, IU/L	64	52	12 - 72
ALK PHOS, IU/dl	3.8	3.4	1.6 - 19
T. Bilirubin, mg/dl	0.64	0.40*	0.6 - .71
BUN, mg/dl	11.2	16.2	5.3 - 22
Creatinine, mg/dl	0.7	0.9	0.46 - 1.1

* Outside 99% confidence limits on normal values.

Subsequently, a study was conducted to evaluate the cardiovascular effect of iv injections of Tween 80 in the dog. Figures 7, 8 and 9 show the effect of 0, 2, 4 or 6 mg/kg of Tween 80 on systolic pressure, diastolic pressure and heart rate, respectively. These results which show induced hypotension at the 2 mg/kg dose were quite surprising because on a volume basis this was a 0.2% solution of Tween 80 and the literature states solutions as high as 10% produce no effect (Gay, 1965). These results are being submitted for publication in an appropriate journal.

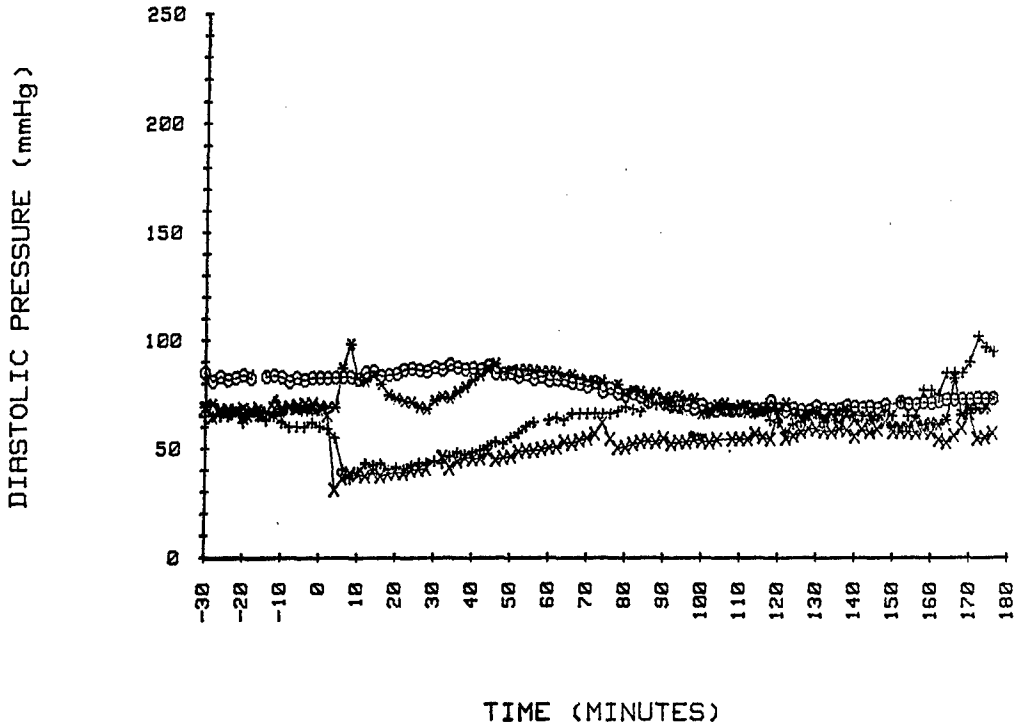


Figure 8. The effect of an iv injection of 10 ml of heparinized saline containing 0 (0), 2 (+), 4 (*) or 6 (X) mg/kg of Tween 80 on diastolic pressure in the dog.

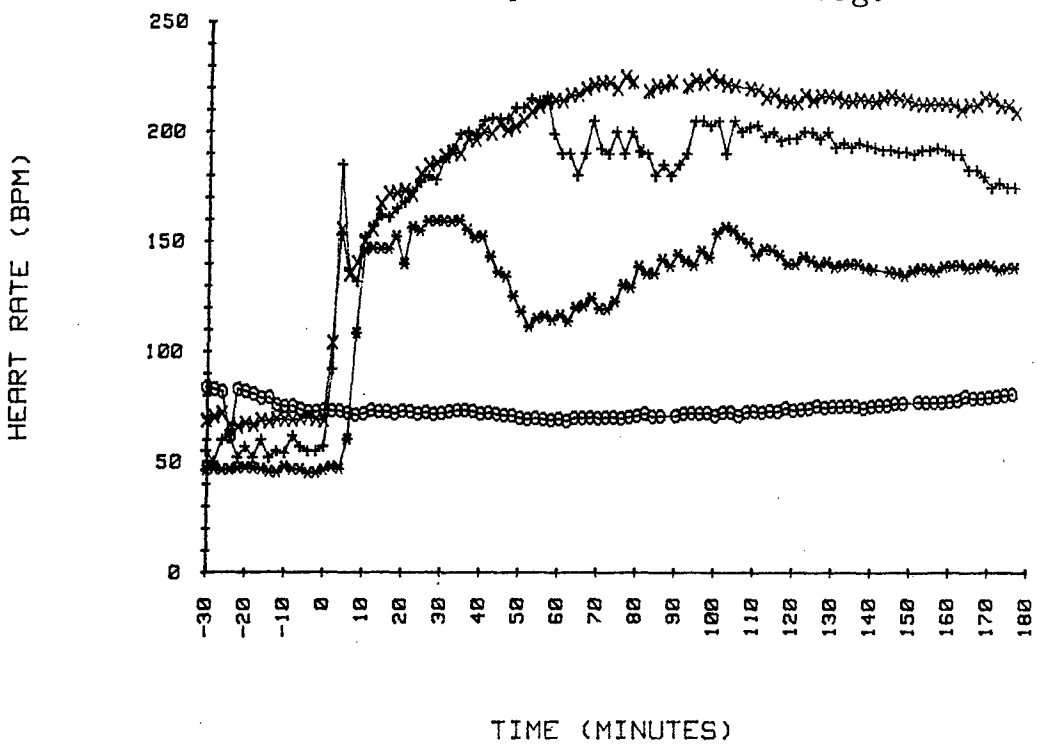


Figure 9. The effect of an iv injection of 10 ml of heparinized saline containing 0 (0), 2 (+), 4 (*) or 6 (X) mg/kg of Tween 80 on heart rate in the dog.

The use of the mass spectrometer for in vivo PGDN analysis has not proven feasible, whereas with a silastic tip arterial catheter and a helium sweep gas it required 17 minutes to get a 90% response time to a step increase in PGDN concentration, the PGDN adhered throughout the system, and it took 2 hours to get a 90% response time to a step decrease in PGDN concentration. Consequently, the indwelling arterial catheter is being used only to monitor Pa_{CO_2} and Pa_{O_2}. The PGDN level is measured in arterial blood samples using gas chromatography with an internal standard. The analog outputs from the mass spectrometer and physiologic transducers have been interfaced to the laboratory's data processor and software written to sample, store in memory, and display current values of variables of choice on a CRT. Figures 1-9 were generated using this software.

EVALUATION OF PULMONARY CLEARANCE IN RATS AFTER EXPOSURE TO OZONE

Introduction

Clearance efficiency of particles deposited in the respiratory system is of major interest when considering pulmonary defense mechanisms. Any decrease in clearance efficiency will consequently increase the retention time of the deposited particles within the respiratory system and, if these particles are toxic or infectious in nature, will increase the susceptibility of attack.

Deposited particles are removed from the respiratory system by three major mechanisms: (1) Particles deposited on the upper ciliated airways, which are lined with a mucous layer, are cleared by cilia propelled movement of this mucous layer up the airways to the pharynx and are swallowed; (2) Particles deposited in the deep nonciliated lung may be engulfed by macrophages and removed using the mucociliary ladder; or (3) Soluble particles may pass through the respiratory membranes into the bloodstream, lymph, or other tissues.

Inhalation of an aerosol of radiolabelled particles deposited throughout the lung provides a method for monitoring pulmonary clearance using external radiation detectors. Measuring the activity excreted in fecal samples taken at fixed intervals monitors the short-term clearance capability via the mucociliary ladder. Measuring thoracic activity monitors the long-term clearance capability of the deep lung. The production of suitably labelled microspheres and the sensitivity of this technique in quantitatively detecting changes in long and short term pulmonary clearance has been previously shown (Hinrichs et al., 1978; Frager et al., 1979; Bianco et al., 1980).

Specific Aims

It is the initial aim of this investigation to document our ability to detect changes in pulmonary clearance in the rat. This would be achieved by repeating a study which has shown that a 4-hour exposure to 1.2 ppm ozone will alter both short and long term pul-

monary clearance. Additionally, we will determine if a simultaneous exposure to CO_2 and the radiolabelled aerosol would increase the deposition due to larger tidal volumes induced by the CO_2 and decrease the intersubject variability in long term clearance.

Methods

Radiolabelled Microspheres

Monodisperse polystyrene latex (PSL) microspheres (Dow Chemical, Midland, MI) labelled with a tightly bound ^{51}Cr isotope (28 day half-life, 0.32 MeV gamma emission) will be obtained from the Department of Community & Environmental Medicine, University of California, Irvine, California using a procedure developed by them (Hinrichs et al., 1978). Batches of a 0.1% suspension of PSL microspheres with a maximum total activity of 100 mCi will be prepared, packaged, and shipped as per regulations via a commercial carrier.

Deposition System

The deposition system is shown in Figure 10. It consists of an aerosol generator and aerosol containment system. A Lovelace-type compressed-air nebulizer (ARIES, Davis, CA) is operated at 40 psig, and the reservoir contains the 0.1% suspension of ^{51}Cr labelled PSL particles with activity of about 30 mCi. After nebulization, the particles are mixed with a diluting air stream for the purpose of drying and limiting the percentage of agglomerates to less than 5%. Breathing air will be used pure or with 4% CO_2 for nebulization and dilution. The air stream with entrained particles is then sent through a column heated to 70°C for additional drying and through a deionization section (TSI, St. Paul, MN) containing a 1 mCi ^{85}Kr source to neutralize any static charge. The aerosol is then introduced into a nose-only inhalation exposure system (Sandia, Albuquerque, NM) which can expose up to 48 rats at a time. The total activity within the chamber will be approximately 250 μCi. The rats are held in 10 inch long, 2.5 inch diameter tubes with adjustable tailgates and air holes to reduce any thermal stress. The aerosol will be characterized using a seven stage cascade impactor (ARIES, Davis, CA).

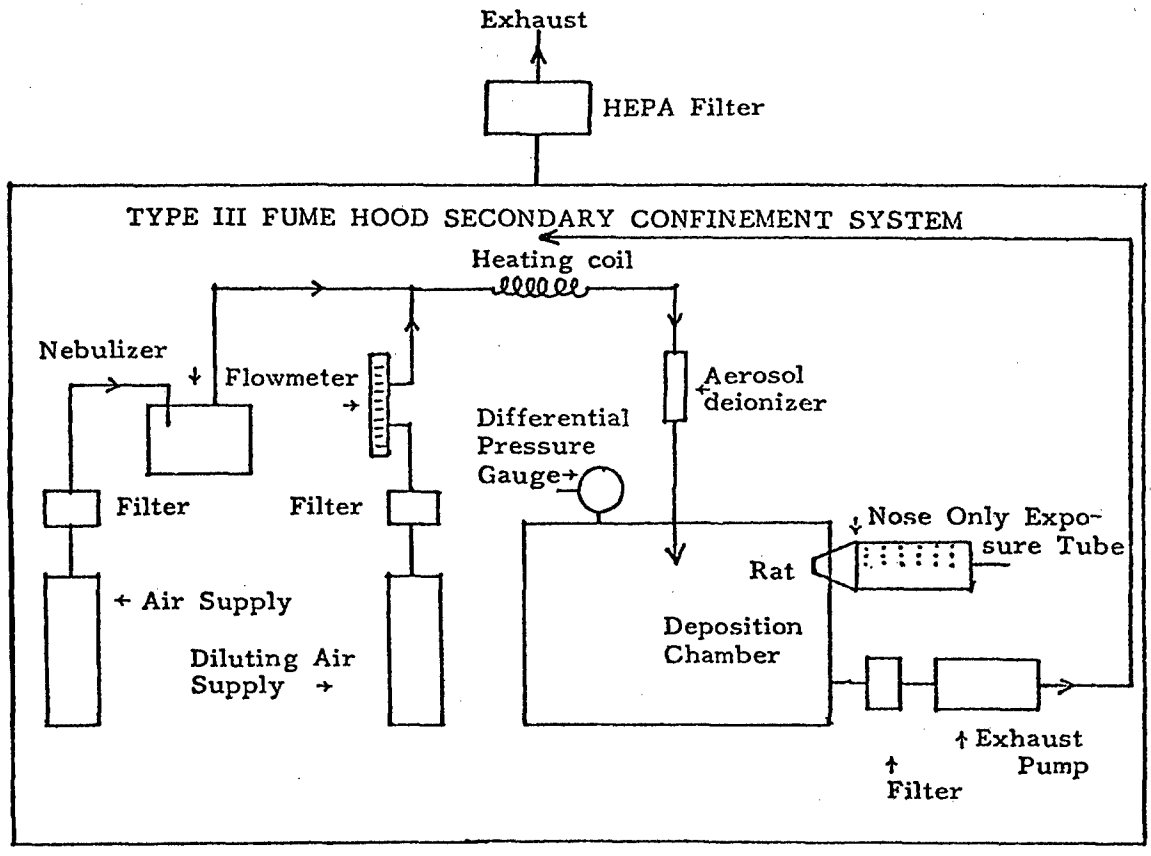


Figure 10. Exposure system for the deposition of ^{51}Cr labelled polystyrene latex microspheres aerosol in rats.

The aerosol is then pulled through filters (Gelman, Ann Arbor, MI) in the chamber exhaust lines to entrap the radiolabelled aerosol for subsequent disposal. Adjustment of the vacuum pump allows the air pressure within the exposure chamber to be held slightly below ambient pressure to prevent loss of the aerosol out through the animal tubes.

The entire exposure system will be contained within a secondary containment system with a downstream HEPA filter to entrap any radiolabelled particles which might escape. After an exposure, the chamber will be purged with a distilled water aerosol and then air for one hour.

Counting System

After particle deposition, the rats are removed from the exposure tubes, and their noses are washed to remove any particles which may be present. Washings are collected in beakers for radiation measurement and appropriate dilution disposal techniques. This minimizes any contamination of the counting system. The rats are then placed in holding tubes and placed within a donut-shaped NaI(Tl) detector shielded by lead and collimated such that radiation

from the respiratory tract is favored for detection. The detector is used with a multichannel analyzer (Model 30, Canberra, Meriden, CT) which integrates counts in the energy region of interest. The initial activity is counted for two 100 second periods and means are calculated. Subsequent measurements will be sufficiently long to make 1000 counts.

The schedule for monitoring thoracic and fecal activity is given in Table 6. The feces are collected in culture tubes from the trap below the individually caged rats and combined with the feces obtained by placing the rats back into the holding tubes which induces defecation. The fecal activity is measured using a NaI(Tl) well detector and measurements are stopped at 48 hours because most of the particles cleared by mucociliary action are excreted by that time.

TABLE 6. RADIOACTIVE COUNTING AND FECAL COLLECTION SCHEDULE USED IN DEPOSITION AND CLEARANCE TESTS IN RATS

Hours Post Deposition	Respiratory Tract Radioactivity Determination	Fecal Collection
0	x	
1-5 Ozone exposure		
5		x
7		x
9		x
11		x
13		x
17		x
21		x
25		x
31		x
37		x
49	x	x
70	x	
142	x	
238	x	
405	x	

Exposure System

Exposures to ozone will occur in a 1 m^3 Rochester type chamber which is maintained at 72 ± 5°F and 50 ± 10% R.H. with an airflow of 32 cfm. The air exposures will occur in a second chamber using the same air supply but without ozone.

Ozone Generation and Monitoring

Ozone will be generated electrically from oxygen using a high voltage silent discharge generator. Total ozone output will be controlled by voltage to maintain the desired concentration of 1.2 ppm. The chamber concentration will be monitored by automatic analytical instrumentation (Technicon) using an iodometric method (Federal Register, 1971).

Exposure Schedule

The radioactive counting and fecal collection schedule is given in Table 6. Four experimental groups are needed to carry out the exposures to a ^{51}Cr aerosol exposure with and without 4% CO_2 and the subsequent exposure to 1.2 ppm ozone or air. In order to detect a 5% change in clearance times, a sample size of 30 is needed in each group. Due to time constraints for measurements, only 15 rats can be used in each group. Therefore, the exposures will have to be replicated.

Histopathology

In order to correlate changes in short and long term lung clearance with morphologic changes, twelve additional rats will be exposed only to the ozone for examination immediately postexposure and 14 days later.

Necropsy

Non-radioactive animals that die or are sacrificed in this study will be necropsied. The necropsy is defined as external examination including body orifices and fixation of the trachea, lung, and heart for histopathologic examination. In addition, tissue will be collected for electron microscopic examination. In order to provide rapid fixation and to maintain the dimensions and configurations of the lung tissue at approximately total lung capacity, the lungs will be fixed by airway perfusion with fixative at a constant perfusion pressure of 30 cm of water for at least 18 hours. A modified Karnovsky's formaldehyde/ glutaraldehyde fixative with added calcium chloride, cacodylic acid, and adjusted to a pH of 7.2 will be used (Nowell and Tyler, 1971; Dungworth et al., 1976).

After fixation, the main bronchus of the left lobe will be bisected longitudinally. A 2-3 mm thick slice will be trimmed from one half for light microscopy (LM). The complementary half is trimmed 4-5 mm thick, and a 0.5 cm square from the juxtahilar end will be taken for scanning electron microscopy (SEM). These two

samples only will have longitudinal sections of bronchus. The remainder of the slice will be minced for transmission electron microscopy (TEM). SEM and TEM will be performed only if analysis of LM results indicates they will be useful. Samples for LM and possible SEM and TEM will also be taken from the anterior and posterior regions of the left lobe.

The extent of collected tissue that will be processed and examined by electron microscopy (SEM and/or TEM) will be determined from the histopathologic observations.

Lung slices for LM will be embedded in paraffin blocks according to standard histologic procedures while samples for SEM will be dehydrated with a graded series of ethyl alcohol solutions and dried in a critical point dryer. The dried blocks of lung tissue will be glued to metal stubs and coated with gold in a vacuum evaporator.

Samples for TEM will be post fixed with 2% osmium tetroxide and dehydrated in a graded series of ethyl alcohol solutions. The tissue is then embedded in epoxy blocks from which thin sections are cut.

Radioactive rats sacrificed at the end of the study will be necropsied. The necropsy for these animals is defined as a gross evaluation of the heart and lung.

Data Analysis

Short-term clearance data are analyzed using log-probit regression, and the time required for each animal to excrete 50% of the total radioactivity excreted through 49 hours is calculated. This term is defined T_S. The delay in short-term clearance (ΔT_S) produced by exposure to air pollutants is then defined as

$$\Delta T_S = (T_{SE} - T_{SC}) \pm \sigma\Delta T_S$$

and

$$\sigma\Delta T_S = (\sigma SE^2 + \sigma SC^2)^{1/2}$$

E = exposed animals
C = control animals
σ = standard deviation
s = short term

The standard error of the mean is then

$$\left[\frac{\sigma_{SE}^2 + \sigma_{SC}^2}{N} \right]^{1/2}$$

where N = number of animals in each group. No corrections for radioactive decay are necessary because all the collected samples are analyzed within a short time for each animal.

Similarly, the long-term clearance data are analyzed using log-linear regression, and an effective clearance halftime of the particles is determined for each animal. This effective halftime (T_E) considers both the biological halftime (T_B) and the physical half-life of the radioactive particles (T_p). T_B is calculated from

$$T_B = \frac{T_E \cdot T_P}{T_P - T_E} \pm \sigma_B$$

where

T_B = biological halftime
T_E = effective halftime
T_P = physical half-life
σ_B = standard deviation of T_B

Using the theory of propagation of errors,

$$\sigma_B = \left[\left[\frac{T_P}{T_P - T_E} + \frac{T_E \cdot T_P}{(T_P - T_E)^2} \right]^2 \cdot s_E^2 \right]^{1/2}$$

where σ_E is the standard deviation of the effective halftimes (T_E).

The change in long-term clearance between exposed and control animals is then defined as ΔT_L where

$$\Delta T_L = (T_{LE} - T_{LC}) \pm \sigma \Delta TL$$

and

$$\sigma \Delta T_L = \left[\sigma_{LE}^2 + \sigma_{LC}^2 \right]^{1/2}$$

E = exposed animals
C = control
L = long term
σ = standard deviation

The standard error of the mean is then

$$\left[\frac{\sigma_{LE}^2 + \sigma_{LC}^2}{N}\right]^{1/2}$$

Statistical tests are performed on the short-term and long-term results using Student's t-test.

Radiation Handling Procedures

Equipment, animals, and samples will all be handled using disposable gloves. Glassware and instruments will be identified for radioisotope laboratory use only. Those items not disposable will be washed in the radioisotope lab sink. Disposable containers, absorbent workbench pads, gloves, and filters will be placed in approved steel drums for disposal as directed by the Base Radiologic Health Physicist. The type and amount of radiation will be recorded. Checks for contamination of laboratory areas will be conducted after each exposure using a GM counter. Film badges will be worn by all laboratory personnel. These procedures have been approved by the Base Radiologic Health Physicist.

Animal Data

Sprague-Dawley male rats (Charles River), 175-225 g, will be used as the experimental animal. The animals will have food and water ad libitum except during the aerosol exposure and subsequent counting periods. Food will be Purina Formula #5008, and the water will be softened. All cage pans will be cleaned daily, and any material contaminated with radiolabelled particles will be disposed of as per regulations. Fecal waste, after counting, will be bagged and disposed of as stated above under Radiation Handling Procedures. Upon completion of the study, the animal carcasses will be stored in a freezer approved for isotope storage and ultimate disposal.

Progress Report

This study has been delayed pending completion by the Engineering Dept. of the containment hood for the radiolabelled aerosol exposure. The containment hood is now in place and has been approved by the Base Radiologic Safety Officer. The lead shielded NaI detectors are in place and the Canberra multichannel analyzer has been interfaced with the laboratory's Hewlett-Packard data processor for transferral and analysis of data.

A pilot study to demonstrate the ability to measure ventilation in a rat using a mask with miniature valves has been concluded. Figures 11, 12 and 13 show the ventilatory response to CO_2 in the rat measured using this mask and the Balzers 511 mass spectrometer. The rat response is seen to be a purely tidal volume increase unlike that of man whose frequency also changes above about 3% CO_2. These data also show that a simultaneous exposure to 4% CO_2 during the radiolabelled aerosol exposure would nearly double the tidal volume and thereby potentially provide a more even distribution of the respired particles throughout the lung. This distribution should reduce the intersubject variability in the clearance of these particles. This hypothesis will be tested as part of the ozone experiment.

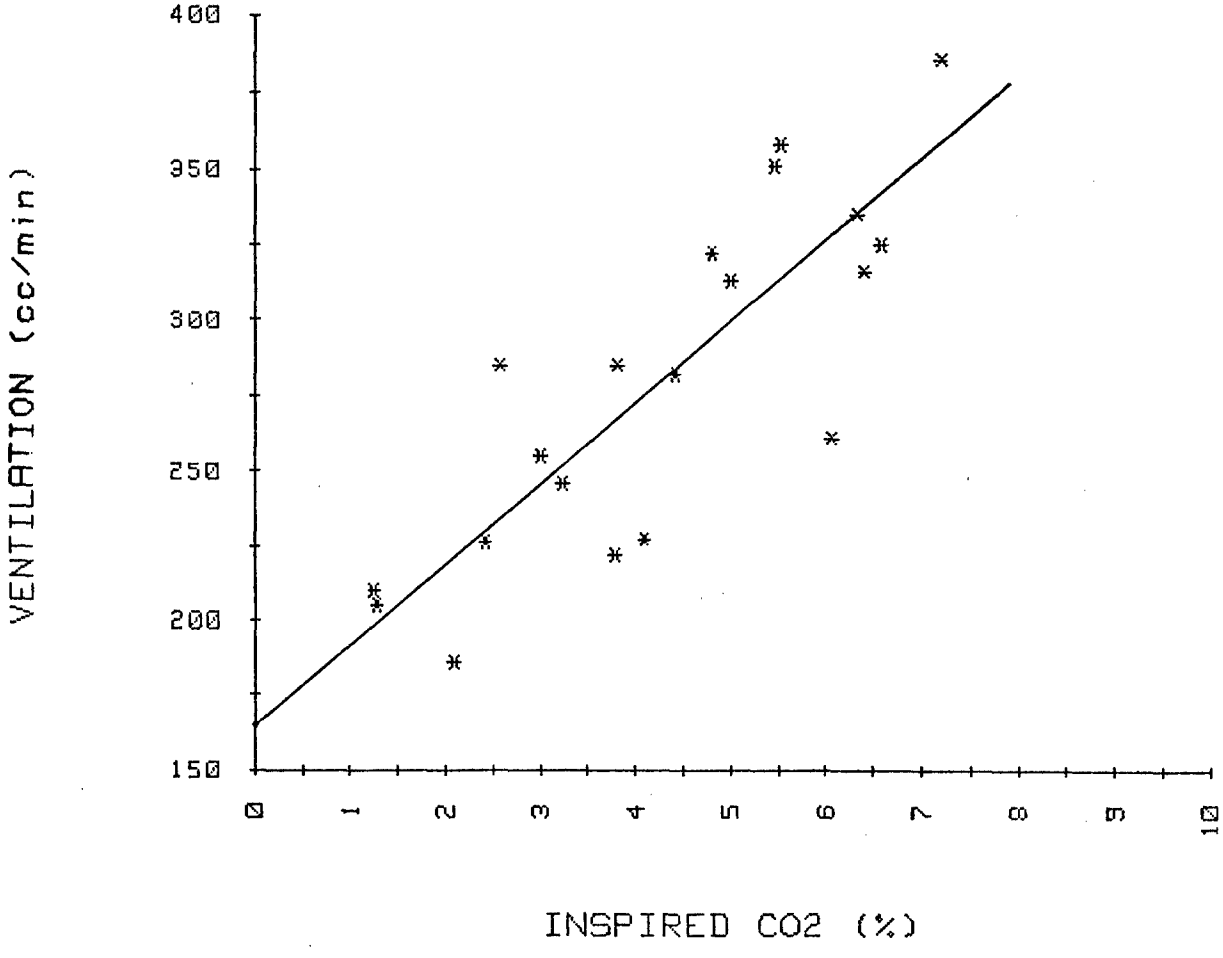


Figure 11. Rat ventilatory response to CO_2 - Ventilation.

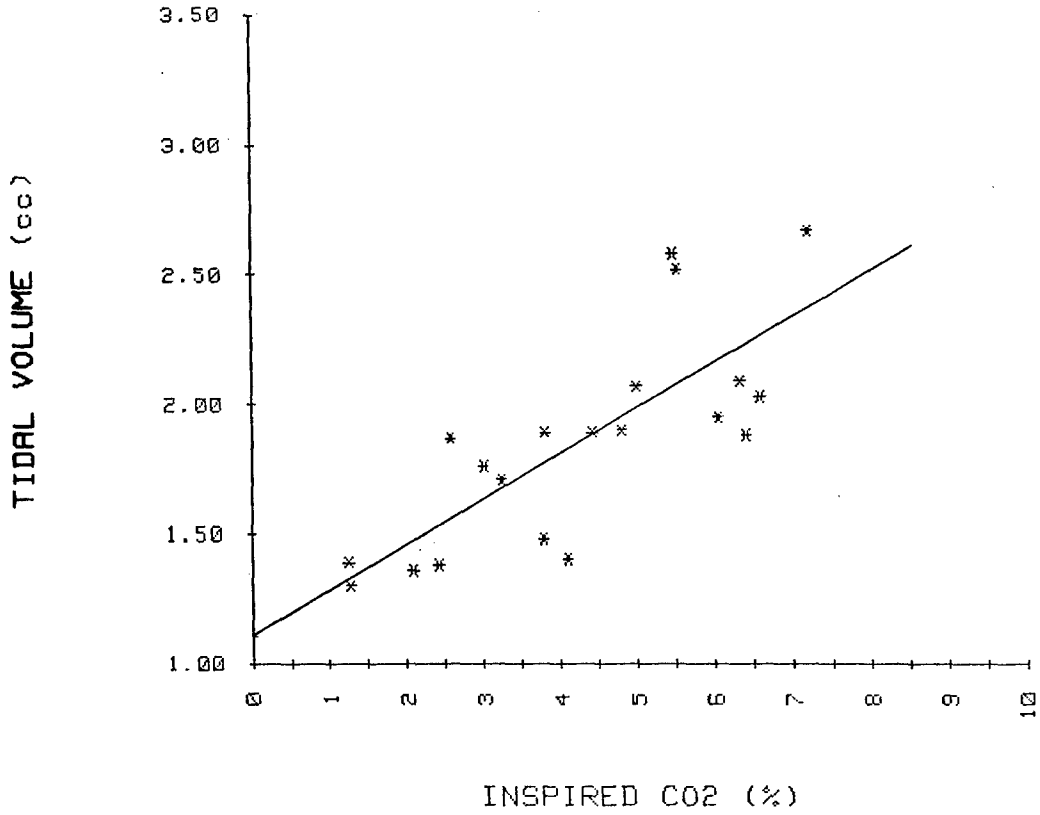


Figure 12. Rat ventilatory response to CO_2 - Tidal Volume.

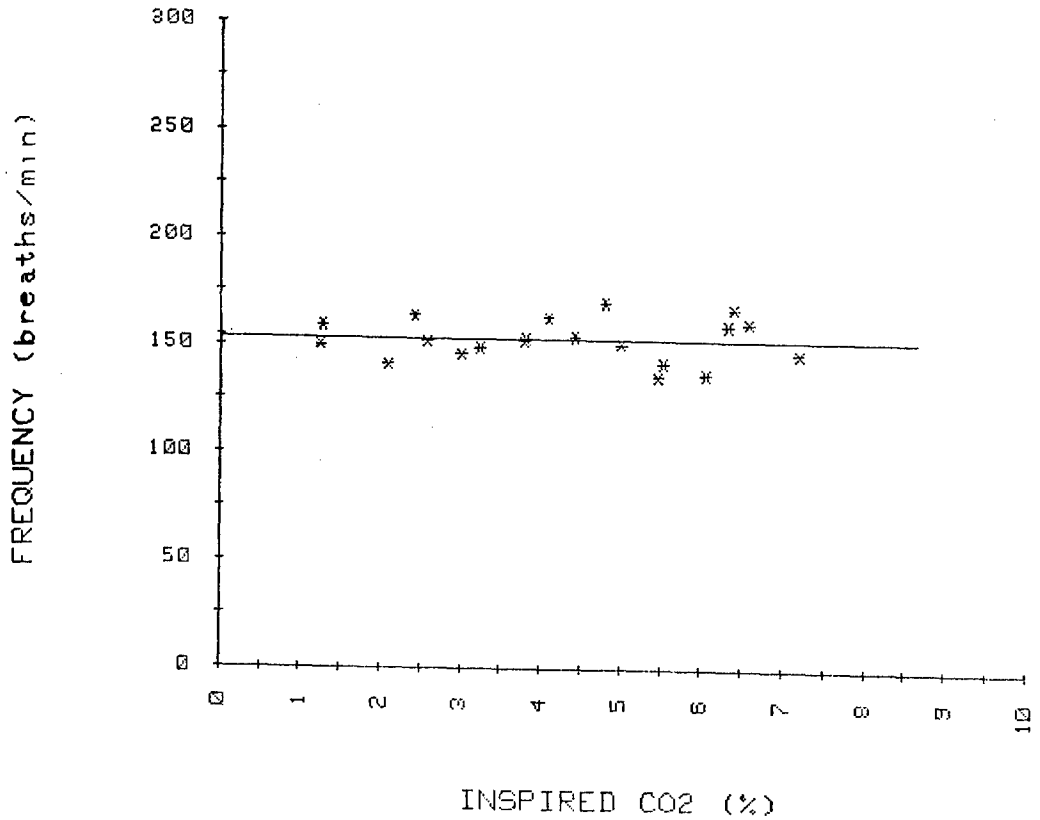


Figure 13. Rat ventilatory response to CO_2 - Frequency.

EVALUATION OF PULMONARY FUNCTION TEST SENSITIVITY TO ENZYME INDUCED EMPHYSEMA IN RATS

Specific Aims

It is the purpose of this investigation to: (1) correlate structure with pulmonary function by determining which pulmonary function test is the most sensitive and correlates best with graded amounts of elastase emphysema in rats; (2) estimate the least amount of emphysema detectable by pulmonary function testing; and (3) provide a baseline for comparison with new pulmonary function tests and their test sensitivity as these tests are developed.

Significance

The detection of a chronic disease such as emphysema at a stage in its development when intervention could substantially improve the prognosis is a goal in prospective medicine. In recent years, a number of new pulmonary function tests have been described which are sensitive to changes in the small airways (e.g., single breath N_2 wash-out, maximum flow-volume maneuver with air or helium-oxygen mixtures). Ideally, the best test would be one which could detect emphysema at its earliest stage or at a point before irreversible damage occurs, i.e., a preemphysematous phase (Thurlbeck, 1979).

Whereas earlier attempts to correlate pulmonary function with structure had proven insensitive (Polecek et al., 1967; Park et al., 1969; Pushpakom et al., 1970; Marco et al., 1972; Niewoehner & Kleinerman, 1972; and Martorana et al., 1973), recent investigators using small airway function tests have associated peripheral airway flow limitation with disorders in these tests (Cosio et al., 1978; Berend et al., 1979; Patty et al., 1980). Inflammation appears to be the best determinant of flow limitation (Cosio et al., 1978). This is of prime importance because of its value in prospective medicine since the inflammation is likely reversible.

Proteolytic enzyme induction of an emphysematous-like disease in the rat has been shown to cause a significant alteration in: (1) total gas volume of the lung (Boyd et al., 1980); (2) functional residual capacity (Johanson and Pierce, 1973; Likens and Mauderly, 1980; Gross and White, 1981; Mauderly, 1981); (3) residual volume (Mauderly, 1981; Gross and White, 1981); (4) carbon monoxide diffusion capacity (Johanson and Pierce, 1973; Mauderly, 1979; Gross and White, 1981; Mauderly, 1981); (5) maximum expiratory flow rates (Gross and White, 1981; Mauderly, 1979; Mauderly, 1981); (6) peak expiratory flow rates (Gross and White, 1981); (7) Partial expiratory flow rates (Mauderly, 1981); and (8) closing volume (Likins and Mauderly, 1980). Similar results have also been found in the hamster and dog (Hayes et al., 1974; O'Neil et al., 1980; Pushpakom, 1970; Koo et al., 1974; Sherter et al., 1974; Fedullo et al., 1980; Lucey et al., 1980; Snider et al., 1977).

While these tests have been found to be sensitive to small airway diseases, these investigations have not been carried out with graded degrees of severity of emphysema.

The proposed investigation would test the ability of these functional procedures in detecting emphysema or a preemphysematous state.

Methods

Morphometric Evaluation of the Lung

Quantification of the amount of emphysema present will be determined by calculating the "mean linear intercept" (Weibel & Gomez, 1962).

Plethysmograph

A whole body pressure or volume plethysmograph has been constructed measuring 4.5" x 4.5" x 10". The outside of the plethysmograph is thermally insulated with 1/4" thick foam. In the pressure mode, the pressure within the plethysmograph is measured using a differential pressure transducer (Validyne, MP45, Northridge, CA). In the volume mode, flow rates are determined by measuring the pressure drop across 4 layers of 400 mesh stainless steel cloth, 0.75" in diameter, and volume is obtained by integration (HP8815A). A system of straight through 3/8" internal diameter, computer controlled solenoid valves (Vacoa, Bohemia, NY) and bidirectional valves (Skinner, New Britain, CT) allow computer controlled (HP9845T) switching of the tracheal catheter to: (1) a respirator (Harvard Model 680, Mills, MA) for animal hyperventilation to produce apnea; (2) positive or negative pressure reservoirs for forced maneuvers; (3) a stopcock for CO diffusion gas mixture insertion and withdrawal; or (4) a flow-through reservoir containing a 4% Halothane:96% O_2 mixture (North American Draeger, Tefford, PA) for anesthesia. The 20 liter positive and negative reservoirs are regulated (Fairchild, Winston-Salem, NC) at +30 and -30 cm H_2O, respectively.

After anesthetizing in a Halothane/O_2 chamber, the rat is intubated using a modified (J. Mauderly, personal communication) 16 gauge intravenous catheter (Cathlon iv, Raritan, NJ). The rat is then placed within the plethysmograph where it breathes the Halothane/O_2 mixture through a stainless steel tube which connects the tracheal cannula through the plethysmograph wall to the valve system outside. A water-filled esophageal catheter is then inserted and transpulmonary pressure is measured using a differential pressure transducer (Validyne, MP45, Northridge, CA) with the reference side connected to the airway via a second water-filled catheter. A Lead II EKG (HP8811D) and rectal temperature (YSI, OH) are recorded.

Box pressure, flow, transpulmonary pressure, flow integrated volume, rectal temperature, EKG, respiratory resistance and compliance are recorded on an 8 channel recorder (HP7758A) and tape recorder (HP3968A) for later feedback for computer (HP9845T) analysis, disk storage (HP7906) or plotting of data (HP9872B).

Resistance and Dynamic Compliance

After intubation, the tracheal tube is attached through the plethysmograph wall to an open circuit 4% Halothane/O_2 mixture and respiration and concurrent anesthesia level allowed to stabilize at 20-30 breaths/minute. The resistance and dynamic compliance as calculated by a respiratory analyzer (HP8816A) using transpulmonary pressure and flow integrated tidal volume are sampled (10 Hz) by the computer and a mean value over 30 seconds is calculated and stored for each variable.

CO Diffusion

After stabilization at 20-30 breaths/minute, to standardize anesthesia level, the rat is hyperventilated to produce apnea and standardize the lung volume history. During the apneic period the lungs are inflated with a gas mixture of 0.5% carbon monoxide (CO), and 0.5% neon (Ne) in nitrogen (N_2). The volume injected with a syringe is that volume previously determined as the difference between FRC (lung volume during apnea) and TLC (by definition, +25 cm H_2O). With the plethysmograph in the volume mode: the flow integrated volume is sampled by the computer; a computer clock times the injection (approximately 8 seconds) from insertion to withdrawal; and the last 2 ml of the withdrawal mixture (alveolar sample) is analyzed by gas chromatography for CO and Ne concentrations. The DLCO is calculated and stored by the computer using standard formulas (Takezawa et al., 1980).

Gas Analysis

Ne and CO concentrations are analyzed using gas chromatography (HP5880). A molecular sieve 5A, 3 mm x 1 meter, stainless steel column is used to separate the gases using helium as the carrier gas at a flow rate of 30 ml/min. Oven temperature is 75°C isothermal.

Quasistatic Pressure Volume Curve

After stabilization of the respiration rate and induction of apnea, a slow inspiration (3 ml/sec) is made to TLC (+25 cm H_2O) and then a slow expiration (3 ml/sec) is made to RV (-25 cm H_2O). The transpulmonary pressure and flow integrated lung volume are sampled by the computer and the slope of the P-V curve between 0 and +10 cm H_2O is calculated and stored. The P-V curve can also be plotted (HP-9872B).

Functional Residual Capacity

After stabilization of the respiration rate, induction of apnea, followed by a 30 second period of breathing, the plethysmograph is switched into the pressure mode and the tracheal tube blocked. As the rat attempts to breathe, the pressure measured volume changes within the plethysmograph and the mouth pressure are sampled by the computer and used to calculate the FRC (lung volume during apnea) using standard formulas (DuBois et al., 1956).

Partial Expiratory Flow-Volume Curve

After stabilization of the respiration rate and then induction of apnea, during the apneic period the rat lung is slowly inflated to +10 cm H_2O. At this point which is approximately 60% TLC, the computer controls a series of maneuvers which switch the animal sequentially to: (1) a -30 cm H_2O reservoir for a forced expiration maneuver to RV; (2) a +30 cm H_2O reservoir for a forced inhalation to TLC; and (3) finally to the -30 cm H_2O reservoir for another forced expiration maneuver. The flow pressure and volume data are sampled by the computer and the solenoid valves are switched at appropriate values to produce this maneuver and also to store and analyze the flow-volume loops. The flow-volume loops are analyzed for forced vital capacity (FVC), forced expired volume in 0.2 seconds (FEV.2, analogous to FEV1 in man), mean maximum expiratory flow rate during 25-75% of expired volume (MMEF25-75%), instantaneous maximum expiratory flow at 50% FVC (MEF50%), peak expiratory flow rate (PEFR), differences between partial and maximal expiratory rate at 60% TLC (PEFV), and the mean transmit time and dispersion of transmit times of the gas being expired during the maneuver (Permutt & Menkes, 1979).

Density Dependent Maximum Expiratory Flow Rate

After stabilization of respiration rate and induction of apnea, during the apneic period a predetermined volume of gas (80% He, 20% O_2) is injected into the lungs to increase the lung volume to TLC (+25 cm H_2O). After this injection, a forced expiration maneuver is performed by switching to the -30 cm H_2O reservoir. The computer sampled flow volume data will be analyzed for comparison of flow rates at 50% of expired volume in the $He-O_2$ mixture versus air. ($\Delta V\ max_{50}$)

Animal Data

Sprague-Dawley male rats (Hilltop Labs.), 175-225 g, will be used as the experimental animal. The animals will have food and water ad libitum. Food will be Purina Formulab #5008, and the water will be softened. All metabolic cages will be cleaned daily.

Progress Report

At the fall 1980 meeting of the Scientific Advisory Board, Dr. Vinegar suggested the frequency response of the plethysmograph needed to be measured at higher frequencies. In order to accomplish this, a loudspeaker pressure generation system as designed by Drs. Jackson and Vinegar was built. A program to generate the sine wave input to the loudspeaker and sample the pressure signals being generated was written. Comparison of gain and phase shift between the outputs from a known Setra pressure transducer and the test transducer have been conducted. Figures 14 and 15 show the effect of 3 types of connectors on the frequency response of the plethysmograph's pressure transducer. The 1/4" diameter tubing will be used in further studies. The plethysmograph's flow transducer is currently being evaluated.

Due to the additional time needed to finish evaluating the frequency response of the plethysmograph, finish developing the software for the pulmonary function testing, and the time needed in the toxicokinetic and pulmonary clearance studies, it is projected that this study will not be conducted until the fall of 1981.

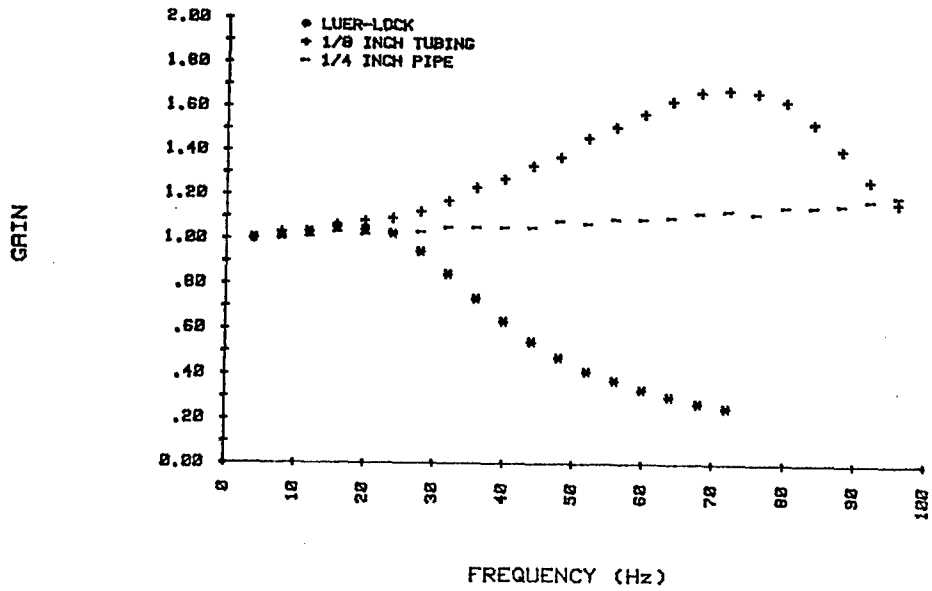


Figure 14. Frequency response of DP45 pressure transducer - Gain.

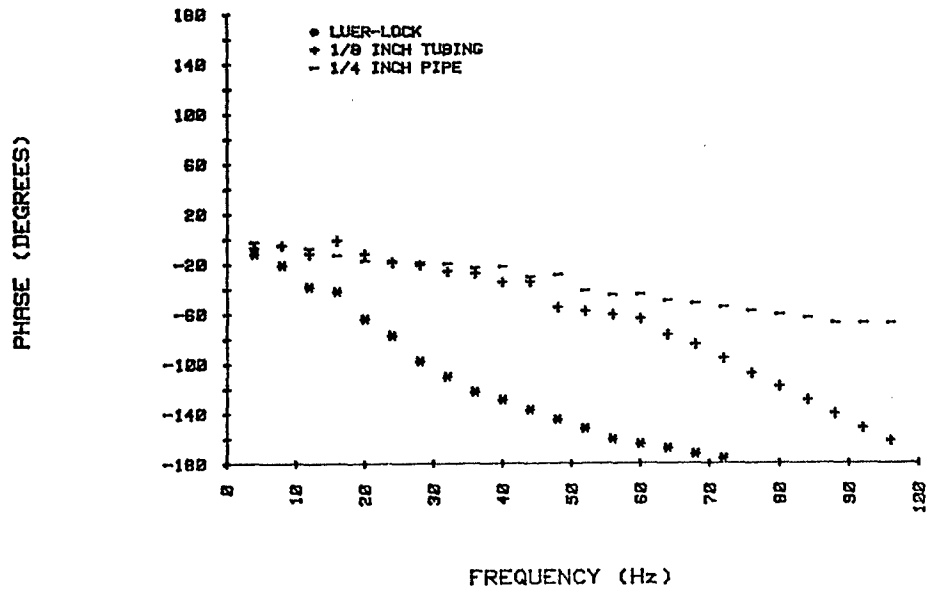


Figure 15. Frequency response of DP45 pressure transducer - Phase.

REFERENCES

Andersen, M.E. and R.A. Smith, (1973), On the mechanism of the oxidation of human and rat hemoglobin by propylene glycol dinitrate, Biochem. Pharmacol., 22:3247-3256.

Berend, N., A. J. Woolcock and G. E. Marlen, (1979), Correlation between the function and structure of the lung in smokers, Am. Rev. Respir. Dis., 119:695-705.

Bianco, A., P. Bassi, M. Belvisi and G. Tarroni, (1980), Inhalation of a radioactively labelled monodisperse aerosol in rats for the assessment of regional deposition and clearance, Am. Ind. Hyg. Assoc. J., 41:563-567.

Boyd, R. L., M. J. Fisher and M. J. Jaeger, (1980), Non-invasive lung function tests in rats with progressive papain-induced emphysema, Respir. Physiol., 40:181-190.

Brain, J. D. and P. A. Valberg, (1979), Deposition of aerosol in the respiratory tract, Am. Rev. Resp. Dis., 120:1325-1373.

Brantigan, J. W., V. L. Gott and M. N. Martz, (1972), A teflon membrane for measurement of blood and intramyocardial gas tensions by mass spectrometry, J. Appl. Physiol., 32:276-282.

Christensen, T. G., A. L. Korthy, G. L. Snider and J. A. Hayes, (1977), Irreversible bronchial goblet cell metaplasia in hamsters with elastase-induced panacinar emphysema, J. Clin. Invest., 59:397-404.

Clark, D. G. and M. H. Litchfield, (1969), The toxicity, metabolism, and pharmacologic properties of propylene glycol 1,2-dinitrate, Tox. Appl. Pharmacol., 15:175-184.

Cosio, M. G., H. Ghezo, J. C. Hogg, R. Corbin, M. Loveland, J. Dosman and P. T. Macklem, (1978), The relations between structural changes in small airways and pulmonary function tests, New Eng. Jour. Med., 298:1277-1281.

Craig, D. K. et al., (1972), Alveolar deposition of $^{239}Pu_{O_2}$ aerosols in beagle dogs as a function of respiration and aerosol parameters, Health Physics, 22:845-856.

DuBois, A. B., S. Y. Botelho, G. N. Bedell, R. Marshall and J. H. Comoroe, Jr., (1956), A rapid plethysmographic method for measuring thoracic gas volume: A comparison with a nitrogen washout method for measuring functional residual capacity in normal subjects, J. Clin. Invest., 35:322-326.

Dungworth, D. L., L. W. Schwartz, W. S. Tyler, and R. F. Phalen, (1976), Morphological methods for evaluation of pulmonary toxicity in animals, Ann. Rev. Pharm. Tox., 16:381-399.

Federal Register, (1971), Vol. 36, No. 84, Friday, April 30, 1971, pp. 3195-8197.

Fedullo, A. J., Y. Jung-Legg, G. L. Snider and J. B. Karlinsky, (1980), Hysteresis ratio: A measure of the mechanical efficiency of fibrotic and emphysematous hamster lung tissue, Am. Rev. Respir. Dis., 122:47-52.

Frager, N. B., R. F. Phalen, and J. L. Kenoyer, (1979), Adaptation to ozone in reference to mucociliary clearance, Arch. Env. Health, 34:51-57.

Fraser, R. T. M. and N. C. Paul, (1968), The mass spectrometry of nitrate esters and related compounds: Part 1, J. Chem. Soc. (B), 6:659-663.

Goldberg, I. S. and R. V. Lourenco, (1973), Deposition of aerosols in pulmonary disease, Arch. Inter. Med., 131:88-91, 1973.

Gross, K. B. and H. J. White, (1981), Non-invasive pulmonary function testing in an elastase-induced model of lung emphysema, Fed. Prog., 117:1-13.

Hayes, J. A., A. Korthy and G. L. Snider (1975), The pathology of elastase-induced panacinar emphysema in hamsters, J. Path., 117:1-13.

Hinrichs, R. J., J. L. Kenoyer, R. F. Phalen, and T. T. Crocker, (1978), Labelling of monodisperse polystyrene microspheres with tightly bound ^{51}Cr, Am. Ind. Hyg. Assoc. J., 39:570-579.

JANNAF Hazards Working Group, (1969), Chemical Rocket/Propellant Hazards, Vol. III, Liquid Propellant Handling, Storage and Transportation, Chemical Propulsion Information Agency, 22-1 to 22-13.

Johanson, W. G. and A. K. Pierce, (1973), Lung structure and function with age in normal rats and rats with papain emphysema, J. Clin. Invest., 52:2921-2927.

Jones, R. A. , J. A. Strickland and J. Siegel, (1972), Toxicity of propylene glycol 1,2-dinitrate in experimental animals, Tox. Appl. Pharmacol., 22:128-137.

Kalin, M. and B. Kylin, (1969), Organic nitrate explosives as monoamino-oxidase inhibitors, Arch. Environ. Health, 18:311-314.

Kessick, M. A., W. G. Characklis, and W. Elvery, (1978), Treatment of wastewater from torpedo refueling facilities, Proc. Ind. Waste Conf., 32:442-449.

Koo, K. W., J. A. Hayes, H. M. Kagen, D. E. Leith, C. Franzblau, and G. L. Snider, (1974), Lung volumes and mechanics following elastase and collagenase in hamsters, Clin. Res., 22:508A.

Kylin, B., A. Englund, H. Ehrner-Samuel and S. Yllner, (1966), A comparative study on the toxicology of nitroglycerine, nitroglycol and propylene glycol dinitrate, Internat. Congress Occup. Health Proc., 15th, 3:191-195.

Likens, S. A. and J. L. Mauderly, (1980), Effects of intratracheally-instilled elastase or histamine on single-breath N_2 washouts in the rat, Physiologist, 23:167A.

Litchfield, M. H., (1971), Aspects of nitrate ester metabolism, J. Pharmaceut. Sci., 60,11:1599-1607.

Lucey, E. C., B. D. Clark, J. B. Karlinsky and G. L. Snider, (1981), Effect of elastase on the volume-pressure relationship of the excise saline-filled lung in young and adult hamsters, Fed. Proc., 40:792.

Lumb, W. V. and E. W. Jones, (1973), Veterinary Anesthesia, Lea & Febiger, Philadelphia, p. 328.

Marco, V., D. R. Meranze, M. Yoshida and P. Kimbel, (1972), Papain-induced experimental emphysema in the dog, J. Appl. Physiol., 33:293-299.

Martorana, P. A., N. W. McKeel, J. W. Richard and N. N. Share, (1973), Function-structure correlation studies on excised hamster lungs in papain-induced emphysema, Can. J. Physiol.: Pharmacol., 51:635-641.

Mauderly, J. L., (1981), Effects of elastase on maximal and partial flow-volume curves of rats, Fed. Proc., 40:385.

Navy Environmental Health Bulletin, (1975), Otto Fuel II, NAVMED P-5112, 10.

Niewoehner, D. and J. Kleinerman, (1972), Physiologic and morphometric studies of experimental emphysema and bronchiolitis in hamsters, Am. Rev. Respir. Dis., 105:997-998.

Nowell, J. A. and W. S. Tyler, (1971), Scanning electron microscopy of the surface morphology of mammalian lungs, Am. Rev. Respir. Dis., 103:313-328.

O'Neil, J. J., R. R. Mercer and J. A. Raub (1980), Pulmonary function: multiple breath nitrogen washout, lung volumes, and diffusing capacity of hamsters with emphysema-like and normal lungs, Am. Rev. Respir. Dis., 121:250A.

Palecek, F., M. Palecekova and D. Aviado, (1967), Emphysema in immature rats: condition produced by tracheal constriction and papain, Arch. Environ. Health, 15:332-342.

Park, S. S., I. P. Goldring, S. C. Shim and M. H. Williams, Jr., (1969), Mechanical properties of the lung in experimental pulmonary emphysema, J. Appl. Physiol., 26:738-744.

Permutt, S. and H. A. Menkes, (1979), Spirometry, analysis of forced expiration within the time domain, The Lung in the Transition Between Health and Disease, (Eds.) P. T. Macklem and S. Permutt, Marcel Dekker, New York, pp. 113-152.

Petty, T. L., G. W. Silvers, R. E. Slanford, M. D. Baird and R. S. Mitchell, (1980), Small airway pathology is related to increased closing capacity and abnormal slope of phase III in excised human lungs, Am. Rev. Respir. Dis., 121:449-456.

Pushpakom, R., J. C. Hogg, A. J. Woolcock, A. E. Angus, P. T. Macklem and W. M. Thurlbeck, (1970), Experimental papain-induced emphysema in dogs, Am. Rev. Respir. Dis., 102:778-789.

Reischel, P., (1979), Personal Communication.

Rodkey, F. L., T. A. Hill, L. L. Pitts and R. F. Robertson, (1979), Spectrophotometric measurement of carboxyhemoglobin and methemoglobin in blood, Clin. Chem., 25,8:1388-1393.

Sherter, C. R., K. W. Koo, J. A. Hayes, D. E. Leith, C. Franzblau and G. L. Snider, (1974), Serial lung volumes and mechanics in elastase-induced emphysema, Clin. Res., 22:718A.

Snider, G. L. and C. B. Sherter, (1977), A one-year study of the evolution of elastase-induced emphysema in hamsters, J. Appl. Physiol.: Respirat. Environ. Exercise Physiol., 43:721-729.

Stewart, R. D., J. E. Peterson, P. E. Newton, C. L. Hake, M. J. Hosko, A. J. LeBrun and G. M. Lawton, (1974), Experimental human exposure to propylene glycol dinitrate, Tox. Appl. Pharmacol., 30:377-395.

Takeyawa, J., F. J. Miller and J. J. O'Neil, (1980), Single-breath diffusing capacity and lung volumes in small laboratory mammals, J. Appl. Physiol.: Respirat. Environ. Exercise Physiol., 48:1052-1059.

Thurlbeck, W. M., (1979), Changes in lung structure, The Lung in the Transition Between Health and Disease, (Eds.) P. T. Macklem and S. Permutt, Marcel Dekker, New York, p. 25.

VanStee, E. W., (July, 1980), Personal communication.

Weibel, E. R. and D. M. Gomez, (1962), A principle for counting tissue structures on random sections, J. Appl. Physiol., 17:343-348.

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